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1. Your reference — LPT0615

2. Patent application number *(The Patent Office will fill in this part)* — 0402728.0

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3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Pharming Ltd.
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Patents ADP number *(if you know it)* 8804023001

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention — PESTICIDES

5. Name of your agent *(if you have one)* — Barker Brettell

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Country	Priority application number *(if you know it)*	Date of Filing *(day/month/year)*

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11. I/We request the grant of a patent on the basis of this application.

Signature Barker Brettell Date 06.02.2004

Barker Brettell

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Yvonne C. Johnson Tel: 0121 456 1364

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PESTICIDES

The present invention relates to the use of fruits, leaves and kernels, in particular loquat kernels, as pesticides, and in particular as nematicides, i.e. compositions that kill nematodes, and more particularly as nematicides that selectively kill plant parasitic nematodes, and to related methods of killing nematodes and related pesticide compositions and their methods of manufacture.

Nematodes are a type of worm like organism. There are different trophic groups of nematodes: fungus feeding, bacteria feeding, predatory and plant parasitic. Plant parasitic nematodes, such as root knot nematodes, are pests because they cause plants to stunt, wilt and/or yellow, often with galls developing over the roots of the plant, due to the feeding of the nematodes on the roots of the plants. Plants that are particularly vulnerable to plant parasitic nematodes include banana, capsicum, carrot, celery, cucurbits, beans, egg fruit, ginger, grape, kiwi fruit, strawberry, lettuce, carnation, chrysanthemum, rose, papaw, passion fruit, pineapple, peach, pumpkin, nectarine and tomato.

There is therefore a need for nematicides to control plant parasitic nematodes; in particular for nematicides that selectively destroy plant parasitic nematodes over other nematodes.

The loquat (*Eriobotrya japonica*) is a fruit of the family Rosaceae. The loquat is also known as the nispero and references hereinafter to the loquat should therefore be read accordingly. The leaves, kernel and fruit of loquat are known for their medicinal uses; in particular the fruit is said to act as a sedative and to relieve coughing and vomiting whilst its leaves and kernel contain amygdalin, otherwise known as laetrile, which is believed by some to have anti-cancer properties. There has not, until

now, however, been any teaching regarding the use of the loquat in pesticidal applications. The family Rosaceae also includes almonds, and the fruits of the genus Prunus, which include apricots, peaches, plums, nectarines and cherries.

The laurel family (Lauracae) includes the avocado, cinnamon, camphor, sassafras, European bay and California bay.

The present invention provides, in a first aspect, the use of the leaves, fruit or kernels of the Rosaceae family or laurel family as pesticides, for example nematicides.

In particular, the use of the kernels of loquat, almond and members of the Prunus genus as pesticides, for example nematicides, is provided.

The use of the leaves and fruits of laurel as pesticides, for example nematicides, is also provided.

In a preferred embodiment, the use of loquat kernels as pesticides is provided.

In a particularly preferred embodiment, the use of loquat kernels as nematicides is provided.

It has been identified by the present inventors that such products, and in particular the loquat kernel, can effectively be used in the killing of pests, in particular nematodes.

In particular, the invention provides the use of loquat kernels as nematicides for plant parasitic nematodes, preferably as selective nematicides for plant parasitic nematodes.

As can be seen from the Examples, selective killing of plant parasitic nematodes can surprisingly be achieved by the application of loquat kernel.

The above fruits, leaves and kernels may be used in any suitable form; in particular they may each be provided in ground, chopped, freeze dried or spray dried form or in the form of an extract. When in the form of an extract any suitable solvent may be used. Preferably the fruits, leaves or kernels are each used in the form of an extract in water.

In a preferred embodiment loquat kernel is used in ground, chopped, freeze dried or spray dried form or in the form of an extract. More preferably the loquat kernel is used in the form of an extract in water.

In one embodiment, all soluble components from the loquat kernel are used, in particular all water soluble components from the loquat kernel may suitably be used.

In one embodiment, the loquat kernel is used in the form of an extract, preferably an extract in water, that has that has a zero or substantially zero concentration of volatiles; in particular the extract may suitably have had all or substantially all volatiles removed.

The loquat kernel may be used in any suitable amounts or concentrations; clearly the amount used should be selected appropriately depending upon the intended application.

Suitably, the kernel may be used in concentrations of 10g/l of loquat kernel or more, preferably 15g/l or more, more preferably 20g/l or more, most preferably 25g/l or more, for example 30g/l or more.

The invention further provides, in a second aspect, a method of killing nematodes, which method comprises providing the leaves, fruit or kernels of the Rosaceae family or laurel family and applying said leaves, fruit or kernels of the Rosaceae family or laurel family to the nematodes or to an area where the nematodes are likely to exist or to an area to where the nematodes are expected to move.

In particular, the leaves, fruit or kernels of the Rosaceae family or laurel family may be the kernels of loquat, almond or members of the Prunus genus or the leaves and fruits of laurel.

Preferably the method of killing nematodes comprises providing loquat kernel and applying the loquat kernel to the nematodes or to an area where the nematodes are likely to exist or to an area to where the nematodes are expected to move.

Suitably the method comprises providing the loquat kernel in ground, chopped, freeze dried or spray dried form or in the form of an extract. When provided in the form of an extract any suitable solvent may be used; however the loquat kernel may in particular be provided in the form of an extract in water. Suitably, all soluble components from the loquat kernel may be provided, in particular all water soluble components from the loquat kernel may suitably be provided. In one embodiment the loquat kernel is provided in the form of an extract, such as an extract in water, from which all or substantially all volatiles have been removed.

The method may suitably comprise the steps of providing loquat kernel, taking an extract thereof and applying the loquat kernel extract to the nematodes or to an area where the nematodes are likely to exist or to an area to where the nematodes are expected to move.

In particular, the step of taking an extract of the loquat kernel may comprise taking an extract of the kernel with water.

The method may further comprise the step of removing all or substantially all volatiles from the extract before the step of applying the extract.

The loquat kernel may be applied in any suitable amounts or concentrations; clearly the amount used should be selected appropriately depending upon the intended application.

Suitably, the kernel is applied at a concentration of 10g/l of loquat kernel or more, preferably 15g/l or more, more preferably 20g/l or more, most preferably 25g/l or more, for example 30g/l or more.

Preferably, the nematodes are plant parasitic nematodes, for example root knot nematodes such as *Meloidogyne* spp.

It is preferred that the method is a method for selectively killing plant parasitic nematodes.

The method may suitably involve applying loquat kernel to a growing medium, such as soil, peat, sand or water, in an area where nematodes are believed to exist or to a growing medium, such as soil, peat, sand or water, in an area to where nematodes are expected to move. For example, the loquat kernel may be applied to or mixed with a growing medium, such as soil, peat, sand or water, that is to have plants planted in it or may be applied to or mixed with a growing medium, such as soil, peat, sand or water, that has plants planted in it, especially when the plants are those that are vulnerable to nematodes.

The invention further provides, in a third aspect, a pesticide comprising the leaves, fruit or kernels of the Rosaceae family or laurel family and a pesticidally acceptable solvent, carrier, excipient or diluent.

In particular, the leaves, fruit or kernels of the Rosaceae family or laurel family may be the kernels of loquat, almond or members of the Prunus genus or the leaves and fruits of laurel.

In a preferred embodiment, the pesticide comprises loquat kernel.

The pesticide may suitably comprise all soluble components of loquat kernel, in particular all water soluble components of loquat kernel.

Suitably, the pesticide comprises 10g/l of loquat kernel or more, preferably 15g/l or more, more preferably 20g/l or more, most preferably 25g/l or more, for example 30g/l or more.

The pesticide may comprise the fruit, leaves or kernel in ground, chopped, freeze dried or spray dried form or in the form of an extract. When in the form of an extract, any suitable solvent may be used. Preferably, the pesticide comprises an extract of loquat kernel in a pesticidally acceptable solvent, carrier, excipient or diluent. Preferably, the pesticide comprises an extract of loquat kernel in water.

In one embodiment, the pesticide comprises an extract of loquat kernel, preferably an extract in water, that has a zero or substantially zero concentration of volatiles. In particular the pesticide comprises an extract of loquat kernel, preferably an extract in water, that has had all or substantially all volatiles removed.

The present invention also provides, in a fourth aspect, a method of producing a pesticide, which method comprises providing ground, chopped, freeze dried or spray dried leaves, fruit or kernels of the Rosaceae family or laurel family.

In particular, the leaves, fruit or kernels of the Rosaceae family or laurel family may be the kernels of loquat, almond and members of the Prunus genus or the leaves and fruits of laurel.

In one embodiment, the method comprises providing ground, chopped, freeze dried or spray dried loquat kernel.

Preferably, the method comprises providing ground loquat kernel and further comprises the step of taking extracts of the ground loquat kernel. Any suitable solvent may be used to take the extract. In particular, the method may comprise the further step of taking extracts in water of the ground loquat kernel.

The method may further comprise the step of removing all or substantially all volatiles from the ground kernel extract.

In one embodiment, the step of providing ground loquat kernel comprises the sub steps of providing loquat kernel and then grinding the kernel.

The invention will now be further described, for the purposes of illustration only, in the following examples.

Examples

Water extracts from Moraira loquat kernels (Algerie or Cardona varieties) were evaluated in a number of experiments. The stock solution used in

each case was prepared by 5 g ground kernels (fresh weight) being extracted in Soxflo™ with 50 ml distilled water.

Example 1 - Experiments with Juvenile root-knot nematodes

The water extracts were tested against plant parasitic nematodes, *Meloidogyne* spp. This root knot nematode is a major crop pest.

Juvenile root-knot nematodes were exposed to different concentrations of the stock solution.

Method:

Two Stock Solutions were prepared as above. One was prepared and stored in the fridge for a month (Stock A) and the other was produced and used immediately (Stock B). Nematodes were then treated with 90, 50 and 10% concentrations of these Stock Solutions (e.g. for a 10% concentration the ratio of stock solution: water was 9:1, v/v). Equivalent controls were left in water.

After treatment the nematodes were placed in water to observe any recovery.

Results:

The product inactivated the nematodes immediately.

Root Knot Nematodes (using Stock B)				
Concentration	% Inactivation after initial application			Average Inactivation(%)
	Replicate 1	Replicate 2	Replicate 3	
90% solution	100	100	100	100.0
50% solution	100	100	100	100.0

10% solution	100	100	99.5	99.8
90% Control	0	0	0	0.00
50% Control	0	0	0	0.00
10% Control	0	0	0	0.00

Root Knot Nematodes (Using Stock A)				
Concentration	% Inactivation after initial application			Average inactivation (%)
90% solution	100	100	100	100.0
50% solution	100	100	100	100.0
10% solution	99	100	100	99.5
90% Control	0	0	0	0.00
50% Control	0	0	0	0.00
10% Control	0	0	0	0.00

When the nematodes were returned to fresh water and observed for 22 hours, only those that had been inactivated by the 10% solution recovered.

Conclusion:

The solutions killed root-knot nematodes at 50% and 90% concentration, but at 10% inactivation was reversible.

Example 2 – Further Experiments with Juvenile root-knot nematodes

Method:

Example 1 was repeated but using 40, 30, and 20% solutions.

Results:

The same results as in Example 1 were obtained. No recovery occurred when nematodes were replaced in water.

Conclusion:

The concentration of Solution at which there is inactivation with no recovery is between 10 and 20%.

In further repeat studies the period of observation of recovery was extended to 8 days (instead of 22 hours as above). No significant recovery was recorded with the 40, 30, and 20% solutions.

Example 3 - The efficacy of the product on inactivation of root-knot nematodes after storage.

Method:

Two different batches of Stock Solution prepared as above were tested to compare whether any loss of efficacy had occurred over time. Stock C was stored in the fridge at 5°C for a month after preparation; Stock D was freshly prepared from ground kernels that had been stored in the freezer before use. The time it took for the root-knot nematodes to become inactivated by the product was compared for the two batches. Two concentrations, 90% and 50%, and a water control were evaluated.

Result:

Both concentrations of both Stock C and Stock D caused instantaneous paralysis to the root-knot nematodes.

Conclusion:

Efficacy was not lost during storage of the product at 5°C.

Example 4 - The role of the volatile compounds in the Moraira kernels

Experiments were carried out to determine whether volatile compounds in the kernels are the principles that kill the nematodes.

Method:

Experiments were conducted under conditions as described above, but in one treatment lids of containers were removed (to allow volatiles to escape). Dosages were 50, 40, 30 and 20% of the above Stock Solution.

Results:

The nematodes were observed over 3 days. In both treatments (with or without lid) and at all concentrations the nematodes ceased to move [indicated by (X)]. Controls remained active (data not shown)

Experiment with lid															
Day	1	2	3	Day	1	2	3	Day	1	2	3	Day	1	2	3
50%	X	X	X	40%	X	X	X	30%	X	X	X	20%	X	X	X

Experiment without lid															
Day	1	2	3	Day	1	2	3	Day	1	2	3	Day	1	2	3
50%	X	X	X	40%	X	X	X	30%	X	X	X	20%	X	X	X

Conclusion:

The results suggest that the active principles are not all volatiles.

The work was then repeated using solutions from which the volatiles had been removed. The same results were shown, which confirms that there are non-volatile compounds in the water extract that will kill root-knot nematodes.

Example 4 - The activity of the water extract against non-plant parasitic nematodes

Experiments were carried out to determine whether the water extracts are active on all types of nematodes, or whether they show some selectivity against different types of nematodes.

Method:

I. Nematodes that feed on bacteria are common soil inhabitants. Some of these nematodes of the genus *Steinernema* were exposed to a 50% solution of the Stock Solution.

II. A field-collected mixed population of all trophic groups of nematodes (fungus-feeding, bacteria-feeding, predatory and plant parasitic) was exposed to a 50% solution of the Stock Solution.

Results:

I. The *Steinernema* nematodes were not inactivated.

II. Of the mixed population all the plant parasites were inactivated. Some of the non-plant parasites were still active, this probably varied across trophic groups.

Conclusion:

Nematodes with different feeding behaviours are affected differently by the product. All plant parasitic nematodes were inactivated.

Example 5 - Activity against larvae of the wax moth *Galleria mellonella*

The wax moth *Galleria mellonella* is a standard laboratory test insect and such moths are readily mass-produced in the laboratory or can be bought from a supplier, e.g. Live Foods Direct.

Methods:

In several studies larvae (larvae either of different stages or only 1st instar larvae) were exposed to the product by direct application. Observations continued over 36 days.

Results:

The product did not show activity when applied to the larvae. Development to adult progressed although in some preliminary tests the water extract appeared to halt development. The experiment as repeated with 1st instar larvae showed no effect.

Example 6 – Activity against some beneficial insects

The wasp *Encarsia formosa*, parasitic on whiteflies, the predacious mite *Phytoseiulus persimilis* that preys on spider mites and nymphs and adults of the thrip predator *Amblyseius cucumeris* (all bought from a biological control company) were exposed to the Stock Solution.

Results:

There was no mortality.

Example 7 - Activity against a pathogenic fungus

Method:

The Stock Solution was tested at full strength against two isolates of the root-infecting fungus *Pythium aphanideratum*.

Plugs of actively growing fungus were placed on water-agar in Petri plates; drops of 25 and 50 microlitres of the product were placed in different positions around the fungus. Growth of the fungus was monitored over 48 h at 25 °C. Sterile water drops were used as controls.

Result:

Growth of the fungus was not inhibited.

CLAIMS

1. The use of the leaves, fruit or kernels of the Rosaceae family or laurel family as pesticides.

2. Use according to Claim 1, wherein the kernels of loquat, almond or members of the Prunus genus or the leaves or fruits of laurel are used as pesticides.

3. Use according to Claim 2, wherein loquat kernels are used as pesticides.

4. Use according to any one of claims 1 to 3, wherein the use is as a nematicide.

5. Use according to Claim 4, wherein loquat kernels are used as nematicides for plant parasitic nematodes.

6. Use according to Claim 5, wherein loquat kernels are used as selective nematicides for plant parasitic nematodes.

7. Use according to any one of the preceding claims wherein the fruits, leaves or kernels are used in ground, chopped, freeze dried or spray dried form or in the form of an extract.

8. Use according to Claim 7, wherein the fruits, leaves or kernels are used in the form of an extract in water.

9. Use according to Claim 7 or Claim 8, wherein loquat kernel is used in the form of an extract that has that has a zero or substantially zero concentration of volatiles.

10. Use according to any one of the preceding claims, wherein all soluble components from the loquat kernel are used.

11. Use according to Claim 10, wherein all water soluble components from the loquat kernel are used.

12. A method of killing nematodes, which method comprises providing the leaves, fruit or kernels of the Rosaceae family or laurel family and applying said leaves, fruit or kernels of the Rosaceae family or laurel family to the nematodes or to an area where the nematodes are likely to exist or to an area to where the nematodes are expected to move.

13. The method of Claim 12 wherein the leaves, fruit or kernels of the Rosaceae family or laurel family are the kernels of loquat, almond or members of the Prunus genus or the leaves or fruits of laurel.

14. The method of Claim 13 wherein the method comprises providing loquat kernel and applying the loquat kernel to the nematodes or to an area where the nematodes are likely to exist or to an area to where the nematodes are expected to move.

15. The method of Claim 14 wherein the method comprises providing the loquat kernel in ground, chopped, freeze dried or spray dried form or in the form of an extract.

16. The method of Claim 14 or Claim 15 wherein all soluble components from the loquat kernel are provided.

17. The method of Claim 15 wherein the loquat kernel is provided in the form of an extract from which all or substantially all volatiles have been removed.

18. The method of any one of claims 12 to 17 wherein the nematodes are plant parasitic nematodes.

19. The method of Claim 18 wherein the nematodes are root knot nematodes.

20. The method of any one of claims 12 to 19 wherein the method comprises applying loquat kernel to a growing medium in an area where nematodes are believed to exist or to a growing medium in an area to where nematodes are expected to move.

21. The method of Claim 20 wherein the loquat kernel is applied to or mixed with a growing medium that is to have plants planted in it or is applied to or mixed with a growing medium that has plants planted in it.

22. A pesticide comprising the leaves, fruit or kernels of the Rosaceae family or laurel family and a pesticidally acceptable solvent, carrier, excipient or diluent.

23. The pesticide of Claim 22 wherein the leaves, fruit or kernels of the Rosaceae family or laurel family are the kernels of loquat, almond or members of the Prunus genus or the leaves or fruits of laurel.

24. The pesticide of Claim 23, which comprises loquat kernel and a pesticidally acceptable solvent, carrier, excipient or diluent.

25. The pesticide of Claim 24, which comprises all soluble components of loquat kernel.

26. The pesticide of Claim 24, which comprises 10g/l of loquat kernel or more.

27. The pesticide of any one of claims 22 to 26 wherein the pesticide comprises loquat kernel in ground, chopped, freeze dried or spray dried form or in the form of an extract.

28. The pesticide of Claim 27 wherein the pesticide comprises an extract of loquat kernel that has a zero or substantially zero concentration of volatiles.

29. A method of producing a pesticide, which method comprises providing ground, chopped, freeze dried or spray dried leaves, fruit or kernels of the Rosaceae family or laurel family.

30. The method of Claim 29, wherein the method comprises providing ground, chopped, freeze dried or spray dried loquat kernel.

31. The method of Claim 30, which comprises providing ground loquat kernel and taking extracts of the ground loquat kernel.

32. The method of Claim 31, which further comprise the step of removing all or substantially all volatiles from the ground kernel extract.